Dr. William Sette
Health Effects Division
OPP

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On August 19, 1999 I received a draft copy of "Office of Pesticide Programs Science Policy on the Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorus and Carbamate Pesticides", dated August 18, 1999, for comment by August 25 of the same year. This document differs remarkably from the last version seen by me. As a consequence, the turn around time needed to respond adequately to the many changes on so complex a subject is inadequate. Furthermore, it comes during my vacation. I will offer comments as best I can under the circumstances, but must reserve until later a more thorough response as may be considered appropriate. This comes somewhat as a surprise to me as I have been long associated with this cholinesterase project, but obviously was not included among the group rewriting this policy.

Before proceeding with a point-by-point comment on this new draft policy paper, there is the need to offer certain preliminary observations. Prior to the June 1997 SAP meeting pertaining to this policy, certain very important questions as to the toxicology (used here in the broadest sense) of cholinesterase inhibition and just how to employ various assessments of cholinesterase inhibition itself in both the assessment of adversity and in the regulatory setting had been left standing with much uncertainty. Drafts of the present developing policy statement recount certain aspects of earlier meetings, i.e. prior to 1997, including those of the SAP. As the result of these lingering uncertainties concerning various scientific aspects, a decision was made within the Agency to address these issues further through a review of the literature, and to draft a policy statement accordingly. I was invited by my management to draft such a review of the scientific aspects. This review, *Dementi (1997)*, hereinafter referred to as the Background Document (1997), went through the normal channels of peer review within the Agency prior to its serving as a supporting document for the policy statement, and was submitted along with the proposed policy and other documents to the June 1997 SAP. The Background Document (1997) covered many topics, which, because of the complexity, cannot be reviewed in this place. These were adequately reflected in the origional draft policy statement delivered to the 1997 SAP. While time does not permit review of the full subject, I should like to mention here two very important topics that appear to be undergoing most substantial revision, or neglect, in this August 18 draft policy with respect to the origional policy statements. Firstly, the Background Document (1997) shed new light on the fact that in the absence of adequate neural cholinesterase inhibition data, it was necessary to rely upon inhibitions of the blood enzymes as surrogates for the missing and/or inadequate neural cholinesterase data. Furthermore, the document served well to explain why neither plasma nor erythrocyte cholinesterase inhibition was preferred or adequate in this regard *until so established through appropriate testing.* In the absence of definitive data showing one or the other of the blood enzymes to be the better correlate or sentinal of neural (CNS, PNS) cholinesterase inhibition, sufficient evidence exists to substantiate that plasma cholinesterase inhibition cannot be arbitrarily singled out as the less relevant of the blood enzymes. Relevance must be established on a compound-by-compound basis under the circumstances of exposure before the more sensitive of the two blood enzymes in any study can be discounted. Rather than attempt to reiterate these findings, I refer the reader to sections from the Background Document (1997) entitled Relevance of Plasma Cholinesterase Inhibition, as this was an important aspect of that

which was submitted to the 1997 SAP.

Secondly, a particularly important aspect of the Background Document (1997**)** is the evidence presented to illustrate that concerns over the toxicological aspects of cholinesterase inhibition go beyond the well characterized and documented litany of clinical symptomatology resulting from acute high level exposures to cholinesterase inhibitors. The Background Document (1997) cites many published works of results from testing in animal models and human epidemology studies that illustrate the potential for long term behavioral or cognitive effects to result from protracted low level exposures to cholinesterase inhibitors, that may not yield classical cholinergic clinical signs or symptoms. This is a very complex subject that I am unable to review here at this time. The reader must refer to the Background Document for the evidence in question. I am not satisfied the latest draft of the policy is true to these concerns.

All of this evidence and reasoning was before the 1997 SAP and received the Panel's approval. In other words, through this entire more recent procedure of review and development of a draft policy statement followed by assessment of the SAP in 1997, the former uncertainties were addressed as intended. Very favorable endorsement for the April 1997 draft policy was rendered by the SAP. Furthermore, the same SAP characterized the Background Document (1997) as excellent. These opinions were rendered by a duly constituted SAP peer review procedure employing outstanding experts in the field, the ultimate in peer review. This more recent August 18 draft of the policy departs in many ways from that which was achieved earlier and endorsed by the SAP. This revised policy itself can claim no such endorsement. It is my perception that the Background Document (1997) is being largely replaced or supplanted by undocumented views rendered in this revised policy statement. Any final policy statement actually should adhere to presentation of policy and not seek through undocumented or inadequately documented discourse to re-develop the science background. That situtation not withstanding, given the great effort reflected in the work presented to the 1997 SAP and SAP's endorsement of the same, I will employ the Background Document (1997) and the many references it cites, as the frame of reference for my point-by-point comments on this latest revised policy statement. Insofar as the use of plasma and erythrocyte cholinesterase inhibition is concerned, I must say up front it becames no longer needful to wrestle with the formerly expressed uncertainiies, but to proceed in the interim (meaning until PNS assays become available) with the use of plasma and erythrocyte cholinesterase inhibition on equal footing, as supported by the SAP (1997).

The following comments constitute my more specific, page-by-page comments on the newly revised August 18, 1999 draft Policy Statement.

1) Under the Executive Summary the draft policy document says: "Of the two common blood measures made, red blood cell ChEI and plasma ChEI, the red blood cell measure may be preferred over plasma because it provides a better representation of the target enzyme." What is the rationale for this assertion? I have seen no actual data establishing this as a generally true statement. One must provide, or at least cite, supporting documentation for such claims. The sole fact that erythrocyte cholinesterase is acetylcholinesterase is not sufficient reason, should that be the reason, to conclude that in <u>in vivo</u> studies it would be expected to emulate more closely than plasma cholinesterase inhibition, neural cholinesterase inhibition. It may tend to be true if the enzymes in their pure forms were isolated from their respective tissues and placed in the same environment, such

as in a solution in a test tube. However, even under that circumstance it may not be true given the fact that there are differing forms of each of the two types of enzymes in various tissues, with widely ranging molecular weights and given that subtle differences in acetylcholine binding specificity may not translate uniformly to the binding of all organophosphate and carbamate inhibitors, as the active site as far as inhibitors is concerned is less restrictive or specific than that of the binding of acetylcholine. Short of actual experimentation, there is little reason to suppose that in <u>in vivo</u> one or the other of the blood enzymes is preferred or adequate as a surrogate for neural cholinesterase inhibition. Certain additional obvious reasons why this is so include, for example, the facts that the half-life of erythrocyte cholinesterase inhibition is contingent upon erythrocyte half-life while that of other cholinesterases depend on other variable factors. Differential inhibitions of cholinesterase occur in differing regions of the CNS, and given this fact erythrocyte cholinesterase inhibition could not serve as an adequate correlate of all brain regional, or sub-regional, choliesterase inhibitions. Indeed plasma cholinesterase inhibition may, in a fortituous way, correlate better with inhibition in one region of the CNS while erythrocyte may serve better in this regard for another region. It is for this latter consideration that the more sensitive of the blood enzymes be used in the absence of adequate neural cholinesterase inhibition data. There are studies showing that erythrocyte cholinesterase inhibition may not even correlate with plasma *acety*cholinesterase inhibition in a given study. Furthermore, as illustrated in the Background Document (1997) there are adequate examples wherein plasma cholinesterase inhibition has been shown to be *superior* to erythrocyte cholinesterase inhibition as a correlate of neural cholinesterase inhibition, such that it cannot be generalized that erythrocte cholinesterase inhibition is the preferred end point. *This must be determined on a compound-by-compound basis under the conditions of exposure.* It would be remiss in our efforts to protect the public health to discount plasma cholinesterase inhibition in the absence of adequate (a big word in this case) neural data, irrespective of the erythrocyte cholinesterase findings. Inhibition of the plasma enzyme establishes the presence of the inhibitor in the system, and cannot be arbitrarly discounted as evidence of a threat to the nervous system, even in the absence of observable clinical signs. For further discussion, see the appended selected pages on the Relevance of Plasma Cholinesterase taken from the Background Document. I should reiterate here that SAP (1997) affirmed this Background Document. I must add that in my opinion it is both a naive assertion and a dangerous public health policy to ascribe greater relevance to erythrocyte cholinesterase inhibition as opposed to plasma cholinesterase inhibition as a correlate or surrogate for neural cholinesterase inhibition absent data on each agent establishing the facts to be otherwise. The arguments in support of this as presented in the Background Document (1997) have been ignored in the draft Policy Statement..

2) On p. 2 the draft policy portends to provide guidance to "....the approaches OPP will take when evaluating cholinesterase inhibition data and functional cholinergic effects.....", where "functional cholinergic effects" as defined in footnote #1 refers to "cholinesterase-related neurophysiological or neurobehavioral clinical signs (or symptoms)". I must say the concerns for cholinergic effects are more broad and complex that those of clinical signs or symptoms, which usually refer to the more obvious effects. By way of illustration, an animal which appears perfectly normal, even to a clinician, may have sever neurochemical compromises in response to cholinesterase inhibition that become most evident only when challenged pharmacologically or by the influence of other stresses which yield no analogous effect in normal untreated animals. The same may be true for symptoms in man. See Persistent Effects Comments in the Background Document (1997) (pp. 12-16). In other words, at

the outset the Policy Statement should acknowledge it will describe approaches that OPP will take in evaluating *all aspects* of compromises to the nervous system, not those limited to clinical signs and symptoms. Indeed, having reviewed this subject, my premier concern at this juncture is for those potential subtle, and not so subtle, effects on behavior that may not be evident as one or more of the constellation of classical cholinergic symptoms in man. I am concerned over effects which even well trained physicians may fail to recognize, that might be manifested in the form of unusual behavior or as reduced, even slightly reduced, performance upon psychometric testing. The scope must not be limited to the most readily observable and recognizable cholinergic clinical signs and symptoms.

3) Section 3.2 "Biology and Toxicology of Cholinesterase-Inhibiting Pesticides" (p. 5) should be removed as this subject is more fully and more adequately developed in the Background Document (1997). Simply say "see Background Document (1997)"

4) The comparisons between butyrylcholinesterase and acetylcholinesterase in the last paragraph of p. 6 are very superficial and the passage neglects to present the remarkable similarities between the analogous six major molecular forms in which both enzymes exist. While it is true that BuChE has no known physiological roles, the same is true of erythrocyte AChE. Also, while it it true that AChE has greater specificity for acetylcholine, it is not necessarily true that binding of cholinesterase inhibitors to the two enzymes is very much different. To the extent this is true, and perhaps irrespective of whether it is true, a host of <u>in vivo</u> parameters will affect the relevance of BuChE versus AChE to neural cholinesterase inhibition such that relevance most be determined in a definitive way under various circumstances of exposure before it can be concluded either blood enzyme is less relevant. This is discussed at length beginning at pages 17 and 79 in the Background Document (1997), again, a review document affirmed as excellent by the SAP (1997).

5) Referring to the draft Policy Statement at p. 7 under 4. reads: Acetylcholinesterase inhibition (AChE) and cholinergic effects resulting .....", should be revised to read *Cholinesterase inhibition and cholinergic effects resulting .....* have long been effective tools in assessing potential risks. Better yet, one might say *Neural acetylcholinesterase inhibition (AChEI) and cholinergic effects resulting....* I say this lest the naive individual be mislead to believe that only acetylcholineserase of the two blood enzymes has a long and historic role in this process.

6) Referring to the penultimate paragraph at p. 7 of the draft Policy, there appears the following statement: "The area of greatest divergence among these reports and in these recommendations involved the interpretation and use of blood measures of ChEI, particularly plasma ChEI for deriving reference doses. Some reviewers and panels placed less (or no) reliance on plasma measures of ChEI and/or less reliance on red blood cell measures of AChEI as a critical effect, than OPP traditionally has placed on each." This is very interesting and important. However, as stated previously, the June 1997 SAP settled the matter. The statement should acknowledge *such historic uncertainty served as the impetus to re-examine the issues, which in turn shed new light on the fact that in the absence of adequate neural cholinesterase inhibition data, it was necessary to rely upon inhibitions of the blood enzymes as surrogates for the missing and/or inadequate neural cholinesterase data. Furthermore, the Background Document (1997) served well to explain why neither plasma nor erythrocyte cholinesterase inhibition was preferred in this regard until so established through appropriate testing. In the absence of definitive data showing one or the other blood enzyme to be*

the better correlate or sentinal of neural cholinesterase inhibition, sufficient evidence has been presented to the effect that plasma is often the preferred blood enzyme, and that plasma cholinesterase inhibition cannot be arbitrarily singled out as less relevant. Again, all of this reasoning received approval of the 1997 SAP. Thus the 1997 SAP was not just one of so many panels to comment on the subject, rather it was the panel that was convened to address the historic confusion over the use of plasma cholinesterase inhibition, as well as other matters. Hence, it became no longer needful to wrestle with the formerly expressed uncertainties, but to proceed in the interim (meaning until PNS assays become available) with the use of plasma and erythrocyte cholinesterase inhibition, whichever proves most relevant in each particular case, or whichever is more sensitive lacking evidence establishing one or the other as the better correlate or sentinal of neural cholinesterase inhibition in a particular case.

7) Referring to the last paragraph at p.7 and over to p. 8 there is some confusion in the draft Policy Statement over reference citations. The document cites US EPA (1997b), but what reference is this in the Bibliography? It is also very disturbing to observe that it is only here that the Background Document (1997) is even mentioned, and only in passing as "a review of the pertinent literature", as if it contributed nothing of noteworthiness, and with respect to earlier policy statements, has even been deleted from the Bibliography.

8) Referring to the first paragraph on p. 8 it is written " The 1997 proposed OPP policy statement differed from previous practice chiefly by ...etc." This paragraph under represents that which transpired at the SAP meeting. "The SAP favorably received this proposal (USEPA, 1997c)". I would suggest adding at this point " and characterized as excellent the Background Document submitted in support of the Policy Statement".

9) In the second paragraph of p. 8, mention is made of the fact that OPP again published the 1997 policy paper for public comment in 1998. This pertained to the Food Quality Protection Act, and presumably was intended in part, at least, to address the requirements of FQPA in relation to the use of cholinesterase data in meeting requirements of this act for both *completeness of data* and its *reliability* where removal of the FQPA imposed 10X safety factor for the protection of infants and children is concerned**.** More needs to be said at this point and elsewhere in this revised Policy Statement pertaining to the relevance of obtaining cholinesterase data in securing evidence of no increased sensitivity of infants and children. It is to be noted that the only basic Guideline testing requirements that have the potential to differentiate sensitivities of adults versus the young are the reproduction and developmental toxicity studies. Yet neither of these studies incorporates cholinesterase assays on adult versus developing individuals. This constitutes a glaring deficiency in the Guideline testing requiremenbts to address the mandates of FQPA to secure protection of the young. The absence of cholinesterase data in these critical studies serves to undermine their adequacy to provide a *reliable* and *complete data base* as mandated by Congress in order to justify removal of the FQPA imposed 10X safety factor. This Policy Statement should acknowledge this concern. Furthermore, there is no mention on p. 10 of the draft Policy Statement of the developmental neurotoxicity study which, if instituted as a Guideline testing requirement, might help alleviate the problem in question.

10) The Conclusions section on p.8 should serve to emphasize why cholinesterase inhibition should

be taken seriously as an adverse effect (difficulty in obtaining other relevant data) and regulated accordingly. It also affirms the need for behavioral (cognitive) effects testing of which there has been little.

11) The Rationale on p. 9 should not be confined to effects resulting from high-level acute exposures, but should note the potential for subclinical effects that may follow longer term low level exposures. See sections in the Background Document (1997) covering persistent effects of cholinesterase inhibition resulting from subchronic and chronic low level exposures to cholinesterase inhibitors.

12) The reference to tolerance on p. 10 does not convey the full implications of tolerance as constituting evidence of adversity. Animals that appear clinically normal in the face of extensive cholinesterase inhibition should not be viewed as normal. This statement should convey the full implications of tolerance as evidence of neurotoxicity. Animals that have become tolerant to extensive cholinesterase inhibition must be viewed as harboring an adverse effect for several reasons: depletion of adaptability, enhanced sensitivity to additional insults of the same or a different kind, extensive changes in neurochemistry, possible presence of subclinical effects that could be demonstrated by the proper testing procedures. Tolerance may serve to explain why animals in various types of studies exhibit differing degrees of clinical signs at equivalent levels of cholinesterase inhibition. See tolerance as discussed in the Background Document (1997).

13) The second full paragraph on p. 11 indicates that sophisticated neurobehavioral test batteries, such as intelligence tests or simple memory tests are rarely if ever used in human studies. It should be emphasized at this point that no animal model would likely be capable of picking up on or detecting cognitive effects that might be manifested in man as a small but meaningful diminution of higher faculties, such as intelligence. This should also serve to underscore as essential the taking of neural cholinesterase inhibition in studies, and relying upon such data as evidence on neurotoxicity. Furthermore, to the extent such data is lacking in human studies, assessments of erythrocyte and plasma cholinesterases, which ever is the more sensitive, should be used as surrogates, indeed as offering the best and only evidence of possible neurtal cholinesterase inhibition in our efforts to protect the public health.

14) In the first full paragraph on p. 13 it is claimed that "...most of the existing data sets will generally contain measures of whole brain AChE, but not....." This statement is not true with respect to the reproduction and developmental toxicity studies, and should be acknowledged. This absence of cholinesterase assays in these particular studies severly compromises the *reliability* of the Guideline testing requirements to address FQPA, and constitute major data gaps. In essence, absent this data, the Guideline testing requirements are fundamentally flawed insofar as their satisfying requirements for deletion of the FQPA imposed 10X safety factor is concerned.

15) On p. 14, mention is made of the development of PNS cholinesterase assays, but not enough is said concerning the status of the project to develop and implement use of the assays. How far away is OPP from obtaining and using such data?

16) On p. 16, mention is made of the fact that the California Department of Health Services requires monitoring of agricultural workers who have contact with highly toxic organophosphorus or

carbamate compounds. I would be curious as to just why such monitoring is confined to the highly toxic cholinesterase inhibitors. The less acutely toxic agents in this class could be as hazardous to humans, perhaps in a more insidious way. This manner of testing is surprising and of concern to me. In this same paragraph, there is no rational basis for use of differing percentages of erythrocyte versus plasma cholinesterase inhibition as a signal to remove workers. This may be entirely appropriate for a particular cholinesterase inhibitor where the differential sensitivity has been worked out, but should not be a generically applied practice.

17) On p. 17, under weight-of-the-evidence considerations, there is no mention of behavioral effects testing or the absence of cholinesteraase data in developmental toxicity and reproduction studies. This compromises the obtaining of *reliable* data for use in the weight-of-the-evidence considerations. This approach, WOE, while sounding good, can be *reliable* only insofar as it incorporates assessments of the critical and most sensitive end points such as cholinesterase inhibition in the case of cholinesterase inhibitors. If cholinesterase data is poor or inadequate, I do not accept that the weight-of-the-*other*-evidence will serve to protect the public health.

18) Near the bottom of p. 17, lip service is given to the need for assessing the potential for differential sensitivity of adult versus young animals (i.e. effects following peri-natal or post-natal exposures) to anticholinesterase chemicals, but there is no acknowledgement that cholinesterase is not assayed in the relevant Guideline studies, so how is the differential sensitivity to be *reliably* evaluated?

19) On p. 18 in the second paragraph there is a repeated effort to discount the relevance of plasma cholinesterase inhibition in comparison with that of erythrocyte cholinesterase inhibition. It is not entirely clear why this affirmation must be repeatedly introduced, but I am inclined to believe perhaps this is the only real purpose of this revised Policy Statement, i.e. to blindly join all other organizations in this misconception.

20) In the 3d paragraph on p. 18, weight-of-the-evidence is mentioned. I cannot over emphasize the fact that weight-of-the-evidence considerations cannot compensate for inadequate cholinesterase data, i.e. either its absence from critical studies or its uselessness because of poor methodology. Something needs to be said here regarding cholinesterase assay methodology and about the status of *reliability* of the data in existing individual pesticide files upon which regulatory end points may be based at the current time.

21) In the last paragraph on p. 18, again there appears the statement that "Each study may include ChE measures in....." As I have stated before, there should be acknowledgement of the absence of such data in those studies critical to the assessment of differential sensitivity of adult and young individuals, namely, the developmental toxicity and reproduction studies. Absent this data there is no justification for deletion of the 10X safety factor imposed under FQPA, weight-of-the-evidence considerations not withstanding.

22) On p. 19 under the topic of Integrative Analysis of the Data Base, concerning the essential elements of a critical study a data base is said to include: "Data on the time course of functional and biochemical effects, including the time of peak effect and the time until complete recovery." How many of the cholinesterase inhibiting pesticides in OPP's files incorporate this data? This particular

data along with that of the other data named in this analysis appear to exceed that which is currently available for most pesticides, and should be acknoweledged as being futuristic.

23) In the 2nd full paragraph on p. 19 should also include “between adult and young individuals”

24) In the last paragraph of p. 19, where is table 1?

25) In the 1st paragraph of p. 20 a claim is made that where erythrocyte cholinesterase assay methodology is reliable, such data is preferred over that of plasma cholinesterase inhibition. How is the judgement to be rendered as to the soundness of cholinesterase methodology? Here once again, the relevance of plasma cholinesterase inhibition is compromised in a generic sense without rationale or scientific justification. I wish to reemphasize all that has been said by me previously.
In this same paragraph it is also written that “.....or the plasma inhibition dose-dependency is more similar to brain AChEI (as well as functional cholinergic effects) than is the .....” Why isn’t it sufficient to say “...is more similar to brain AChEI” *absent the parenthetical qualifying statement*? Suppose, for example, plasma cholinesterase inhibition proves the superior correlate of brain cholinesterase inhibition in animal models, in the absence of “functional cholinergic effects” wherein these effects have been inadequately pursued and wherein brain cholinesterase inhibition in humans is associated with unusual behavior or other subclinical cognitive effects, would this policy paper discount plasma cholinesterase inhibition as a regulatory end point for human exposures?
26) In the 1st paragraph of p.21 there is the statement: “ In response to comments received on the 1997 OPP policy paper, this guidance places more emphasis on RBC ChEI measures compared to plasma measures”. Given the great importance of this particular subject, and the considerable effort expended in the Background Document (1997) to evaluate the subject, a presentation in the policy statement of more than an affirmation of comments received in opposition to the origional policy is indicated. Let the public see what these statements were, including something of their rationale or experimental basis. For example, was there possibly any research data submitted on a substantial number of cholinesterase inhibitors illustrating superior correlations between erythrocyte (as contrasted with plasma cholinesterase) and neural cholinesterase inhibition, to stand in support of such a generic statement? As a member of the cholinesterase team, I have not seen any such data. Please let me see the data. Until such data has been received and fully evaluated, there is no justification for changing the origional policy statement, particularly since it was endorsed by peer review of the SAP (1997), nor is there any refutation of the presentation on the Relevance of Plasma Cholinesterase Inhibition in the Background Document (1997). To the extent the policy is changed without evidence to support the change, a mockery is being made of the official activities of the FIFRA Scientific Advisory Panel in its dealing with complex and important subjects. In this instance, in reversing the guidance offered by the SAP, has OPP sought concurrence for the change from the same panel of experts?

27) In the 1st paragraph on p. 21, I could concur with the notion that a two-fold difference in NOELs might be regarded as small, but not five or even three. The more fundamental question is the reliability of the data base for cholinesterase inhibition for all cholinesterase inhibitors, particularly in view of the FQPA requirements for *complete* and *reliable* data for the protection of the Nation’s youth.

I appreciate being accorded the opportunity to comment of this draft policy statement.

Brian Dementi, Ph.D., DABT
Toxicologist
Health Effects Division